4,367,146. A plug-flow is in effect a flow-through bioreactor. A bioreactor plug is a plug used in a bioreactor. It is identified in Figure 1, which schematically shows a plug-flow bioreactor, by reference numeral 5. A bioreactor that includes the plug inherently comprises all the features of the plug itself. So, if the plug is said to include a matrix, the bioreactor is said to include a matrix, etc. The plug has an outlet and an inlet which connect to an inlet and an outlet of the bioreactor, respectively.

In view of the above, Applicant believes to have overcome the Examiner's objections.

PCT Article 33(2) - Sussman et al.

The examiner has stated that claims 89-99 lack novelty under PCT Article 33(2) as being anticipated by Sussman et al. Claims 89-99 have now been canceled. Nevertheless, the claimed plug is said to include (as opposed to "to be able to support) 5×10^6 stromal cells per cubic centimeter of substrate. Sussman et al. clearly fails to teach a plug that includes 5×10^6 stromal cells per cubic centimeter of substrate. It can be interpreted that Sussman et al. teach a plug including 5×10^5 cells per cubic centimeter of substrate, which is an order of magnitude less cells. Thus, Sussman et al. clearly failed to realize the potential of their plug to support stromal cells.

PCT Article 33(3)

Naughton et al. in view of Sussman et al. and Stephanopouos et al.

The examiner has stated that claims 1-20 and 51-70 lack an inventive step under PCT Article 33(3) as being obvious over Naughton et al. in view of Sussman et al. and Stephanopouos et al. The Examiner statements are respectfully traversed.

The Examiner points out that Naughton et al. disclose growing stromal cells on a three-dimensional matrix and inoculating same with stem cells, such as

hematopoietic stem cells. The Examiner further points out that Sussman et al. and Stephanopouos et al. both teach a plug-flow bioreactor. The examiner concludes that it would have been obvious to use the matrix of Naughton et al., the non-woven fibrous sheet packed in a column for cell culture disclosed by Sussman et al. to obtain a flow-through reactor having an inlet and outlet as suggested by Sussman et al. and Stephanopouos et al. since such a reactor would have been expected to provide advantages of a beneficial environment for cell culture and continuous flow.

Naughton et al., discloses the growth of bone marrow stromal cells on a three dimensional matrix, followed by the inoculation of the stromal matrix with bone marrow cells, thereby allowing the long-term maintenance of hematopoietic cells.

Naughton et al. state that "stem cell replication in this system can be inferred from the sustained proliferation of committed progenitors" (column 21, line 26). They also state that the system "appears to maximize the proliferation of multipotential hematopoietic stem cells which have the capacity of repopulating bone marrow..." (column 21 line 3).

Reviewing the experimental data presented by Naughton et al. in Figures 3 and 4, it is clearly evident that the three dimensional system disclosed thereby appears to maintain physiological numbers/levels and distribution of hematopoietic cells. This is in complete contrast with the present invention as claimed in claims 1 and 51, in which a three dimensional stromal cell bioreactor provides a system for the expansion of transplantable human hematopoietic stem cells, while maintaining an undifferentiated population of stem cells (i.e., differentiationless stem cell expansion).

Naughton et al. fail to teach or suggest differentiationless stem cell expansion, whereas the claimed invention is clearly limited to differentiationless stem cell expansion.

Thus, one of skills in the art interested in obtaining stem cell expansion would not be motivated to rely on the teachings of Naughton et al. altogether. One

interested in differentiationless stem cell expansion would be reluctant to use the teaching of Naughton et al. which disclose the exact opposite, i.e., how to maintain a differentiated cell population. Needless to say the one of skills in the art interested in obtaining differentiationless cell expansion would not be motivated to combine the teachings of Naughton et al. with those of Sussman et al. and/or Stephanopouos et al. because either of these references fails to teach differentiationless cell expansion. Also, it is clear that one of skills in the art, based on the teachings of Naughton et al., Sussman et al. and Stephanopouos et al., taken alone, or in any combination, cannot reasonably expect to obtain differentiationless stem cell expansion.

Naughton et al. show a capacity to merely mimic the physiological events occurring within the bone marrow. The utility of the system described by Naughton et al. for the purpose of *ex vivo* cell expansion (cell proliferation devoid of differentiation) prior to stem cell transplantation, is clearly not disclosed nor is it suggested.

The method described by Naughton et al. involves the seeding of bone marrow cells on a pre-established 3D stromal cell matrix. Since the inoculum used by Naughton et al. also contains stromal cell elements and/or precursors, Naughton et al. cannot, via their experimentation, distinguish between an effect of the 3D stromal cell matrix or of the co-inoculated stromal cells, on the output of hematopoietic cells. This leaves the claim of an effect of the 3D stromal matrix, uninvestigated and questionable.

Thus, yet another difference between the claimed invention and Naughton et al. is that according to the claimed invention stem cells are grown in plug-flow bioreactor in which a three dimensional stromal cell culture has been pre-established.

Naughton et al. state that "where the stem cells or reserve cells can be readily isolated, these may be used to preferentially inoculate the three dimensional stromal support" (column 15 line 57). This hypothesis was put to test by the inventors of the invention disclosed by the instant application.

Clear evidence was collected revealing that a static 3D stromal cell culture system (as described by Naughton et al.) is highly unsuitable for the purpose of stem cell expansion. As shown in Figure 1 below, 7 days following seeding of isolated cord blood CD34+ cells onto a bioreactor containing a preestablished human marrow stromal matrix, an approximate 2-fold expansion of transplantable HSCs (i.e. CD34+38-CXCR4+ cells) can be seen, with circulating and carrier-attached CD34+38-CXCR4+ representing 63 % and 37 % of total bioreactor yield, respectively. While stromal cell-coated carriers within the bioreactor support 89 % of input CD34+38-CXCR4+ cells, parallel 7-day cultures of carriers which had been removed from the bioreactor and maintained in static cultures, can support only 10 % of seeded CD34+38-CXCR4+ cells. These findings clearly indicate the essential role of the use of a flow bioreactor for the differentiationless expansion of transplantable stem cells.

Thus, based on the combined teachings of Naughton et al., the teachings of the present invention and the teachings of Figure 1 herein one can conclude the following. When grown in a static bioreactor, stromal cells fail to condition the bioreactor's medium, so as to allow differentiationless stem cell expansion (Figure 1), yet they can condition the bioreactor's medium, so as to allow maintenance of a differentiated cell population (Naughton et al.). When grown in a flow through bioreactor, stromal cells manage to condition the bioreactor's medium, so as to allow differentiationless stem cell expansion (the present invention).

Hence, it is clear that since Naughton et al. fails to teach differentiationless stem cell expansion, rather Naughton et al. teach maintenance of a differentiated cell population, Naughton et al. creates no expectation to succeed in obtaining differentiationless stem cell expansion, using either static bioreactor, as taught by Naughton et al. themselves, or flow through bioreactor, as taught by Sussman et al. and Stephanopouos et al.